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(54) **LETTUCE CULTIVAR GREEN THUNDER**

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800/279; 800/298; 800/300; 800/301; 800/302;
800/303; 800/260

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ABSTRACT

A novel romaine lettuce cultivar, designated Green Thunder, is disclosed. The invention relates to the seeds of lettuce cultivar Green Thunder, to the plants of lettuce cultivar Green Thunder and to methods for producing a lettuce plant by crossing the cultivar Green Thunder with itself or another lettuce cultivar. The invention further relates to methods for producing a lettuce plant containing in its genetic material one or more transgenes and to the transgenic plants produced by that method and to methods for producing other lettuce cultivars derived from the cultivar Green Thunder.

26 Claims, No Drawings

LETTUCE CULTIVAR GREEN THUNDER

BACKGROUND OF THE INVENTION

The present invention relates to a new and distinctive Romaine lettuce (*Lactuca sativa*) cultivar, designated Green Thunder. All publications cited in this application are herein incorporated by reference.

There are numerous steps in the development of any novel, desirable plant germplasm. Plant breeding begins with the analysis and definition of problems and weaknesses of the current germplasm, the establishment of program goals, and the definition of specific breeding objectives. The next step is selection of germplasm that possess the traits to meet the program goals. The goal is to combine in a single variety or hybrid an improved combination of desirable traits from the parental germplasm. These important traits may include increased head size and weight, higher seed yield, improved color, resistance to diseases and insects, tolerance to drought and heat, and better agronomic quality.

Practically speaking, all cultivated forms of lettuce belong to the highly polymorphic species *Lactuca sativa* that is grown for its edible head and leaves. As a crop, lettuces are grown commercially wherever environmental conditions permit the production of an economically viable yield. Lettuce is the world's most popular salad. In the United States, the principal growing regions are California and Arizona which produce approximately 287,000 acres out of a total annual acreage of more than 300,000 acres (USDA, 2001). Fresh lettuces are available in the United States year-round although the greatest supply is from May through October. For planting purposes, the lettuce season is typically divided into three categories, early, mid and late, with the coastal areas planting from January to August, and the desert regions from August to December. Fresh lettuces are consumed nearly exclusively as fresh, raw product, occasionally as a cooked vegetable.

Lactuca sativa is in the Cichoreae tribe of the Asteraceae (Compositae family). Lettuce is related to chicory, sunflower, aster, dandelion, artichoke and chrysanthemum. *Sativa* is one of about 300 species in the genus *Lactuca*. There are seven different morphological types of lettuces. The Crisphead group includes the iceberg and batavian types. Iceberg lettuce has a large, firm head with a crisp texture and a white or creamy yellow interior. Batavian lettuce predates the iceberg type and has a smaller and less firm head. The Butterhead group has a small, soft head with an almost oily texture. Romaine lettuce, also known as cos lettuce, has elongated upright leaves forming a loose, loaf shaped head. The outer leaves are usually dark green. The Leaf lettuces come in many varieties, none of which form a head. The next three types are seldom seen in the United States: Latin lettuce looks like a cross between romaine and butterhead; stem lettuce has long, narrow leaves and thick, edible stems, and Oilseed lettuce is a type grown for its large seeds that are pressed to obtain oil.

Lactuca sativa is a simple diploid species with nine pairs of chromosomes. Lettuce is an obligate self-pollinating species. This means that the pollen is shed before stigma emergence, assuring 100% self-fertilization. Since each lettuce flower is an aggregate of about 10-20 individual florets (typical of the Compositae family), manual removal of the anther tubes containing the pollen is tedious. As such, a modified method of misting to wash the pollen off prior to fertilization is needed to assure crossing or hybridization. About 60-90 min past sunrise, flowers to be used for crossings are selected. The basis for selection are open

flowers, with the stigma emerged and the pollen visibly attached to the single stigma (about 10-20 stigma). Using 3-4 pumps of water from a regular spray bottle, the pollen grains are washed off with enough pressure to dislodge the pollen grains, but not enough to damage the style. Excess water is dried off with clean paper towels. About 30 min later the styles should spring back up and the two lobes of the stigma are visibly open in a "V" shape. Pollen from another variety or donor parent is then introduced by gently rubbing the stigma and style of the donor parent to the maternal parent. Tags with the pertinent information on date and pedigree are then secured to the flowers

Choice of breeding or selection methods depends on the mode of plant reproduction, the heritability of the trait(s) being improved, and the type of cultivar used commercially (e.g., F₁ hybrid cultivar, pureline cultivar, etc.). For highly heritable traits, a choice of superior individual plants evaluated at a single location will be effective, whereas for traits with low heritability, selection should be based on mean values obtained from replicated evaluations of families of related plants. Popular selection methods commonly include pedigree selection, modified pedigree selection, mass selection, and recurrent selection.

The complexity of inheritance influences choice of breeding method. Backcross breeding is used to transfer one or a few favorable genes for a highly heritable trait into a desirable cultivar. This approach has been used extensively for breeding disease-resistant cultivars. Various recurrent selection techniques are used to improve quantitatively inherited traits controlled by numerous genes. The use of recurrent selection in self-pollinating crops depends on the ease of pollination, the frequency of successful hybrids from each pollination, and the number of hybrid offspring from each successful cross

Each breeding program should include a periodic, objective evaluation of the efficiency of the breeding procedure. Evaluation criteria vary depending on the goal and objectives, but should include gain from selection per year based on comparisons to an appropriate standard, overall value of the advanced breeding lines, and number of successful cultivars produced per unit of input (e.g., per year, per dollar expended, etc.). Promising advanced breeding lines are thoroughly tested and compared to appropriate standards in environments representative of the commercial target area(s) for three years at least. The best lines are candidates for new commercial cultivars; those still deficient in a few traits are used as parents to produce new populations for further selection. These processes, which lead to the final step of marketing and distribution, usually take from eight to 12 years from the time the first cross is made. Therefore, development of new cultivars is a time-consuming process that requires precise forward planning, efficient use of resources, and a minimum of changes in direction.

A most difficult task is the identification of individuals that are genetically superior, because for most traits the true genotypic value is masked by other confounding plant traits or environmental factors. One method of identifying a superior plant is to observe its performance relative to other experimental plants and to a widely grown standard cultivar. If a single observation is inconclusive, replicated observations provide a better estimate of its genetic worth.

The goal of lettuce plant breeding is to develop new, unique and superior lettuce cultivars. The breeder initially selects and crosses two or more parental cultivars, followed by repeated selfing and selection, producing many new genetic combinations. The breeder can theoretically generate billions of different genetic combinations via crossing,

selfing and mutations. The breeder has no direct control at the cellular level. Therefore, two breeders will never develop the same cultivar, or even very similar cultivars, having the same lettuce traits.

Each year, the plant breeder selects the germplasm to advance to the next generation. This germplasm is grown under unique and different geographical, climatic and soil conditions and further selections are then made, during and at the end of the growing season. The cultivars that are developed are unpredictable because the breeder's selection occurs in unique environments, with no control at the DNA level (using conventional breeding procedures), and with millions of different possible genetic combinations being generated. A breeder of ordinary skill in the art cannot predict the final resulting cultivars he develops, except possibly in a very gross and general fashion. The same breeder cannot produce the same cultivar twice by using the exact same original parents and the same selection techniques. This unpredictability results in the expenditure of large research monies to develop superior lettuce cultivars.

The development of commercial lettuce cultivars requires the development of lettuce varieties, the crossing of these varieties, and the evaluation of the crosses. Pedigree breeding and recurrent selection breeding methods are used to develop cultivars from breeding populations. Breeding programs combine desirable traits from two or more varieties or various broad-based sources into breeding pools from which cultivars are developed by selfing and selection of desired phenotypes. The new cultivars are crossed with other varieties and the hybrids from these crosses are evaluated to determine which have commercial potential.

Pedigree breeding is used commonly for the improvement of self-pollinating crops or inbred cultivars of cross-pollinating crops. Two parents which possess favorable, complementary traits are crossed to produce an F_1 . An F_2 population is produced by selfing one or several F_1 s or by intercrossing two F_1 s (sib mating). Selection of the best individuals is usually begun in the F_2 population; then, beginning in the F_3 , the best individuals in the best families are selected. Replicated testing of families, or hybrid combinations involving individuals of these families, often follows in the F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (i.e., F_6 and F_7), the best cultivars or mixtures of phenotypically similar cultivars are tested for potential release as new cultivars.

Mass and recurrent selections can be used to improve populations of either self- or cross-pollinating crops. A genetically variable population of heterozygous individuals is either identified or created by intercrossing several different parents. The best plants are selected based on individual superiority, outstanding progeny, or excellent combining ability. The selected plants are intercrossed to produce a new population in which further cycles of selection are continued.

Backcross breeding has been used to transfer genes for a simply inherited, highly heritable trait into a desirable homozygous cultivar or line that is the recurrent parent. The source of the trait to be transferred is called the donor parent. After the initial cross, individuals possessing the phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The resulting plant is expected to have the attributes of the recurrent parent (e.g., cultivar) and the desirable trait transferred from the donor parent.

The single-seed descent procedure in the strict sense refers to planting a segregating population, harvesting a

sample of one seed per plant, and using the one-seed sample to plant the next generation. When the population has been advanced from the F_2 to the desired level of inbreeding, the plants from which cultivars are derived will each trace to different F_2 individuals. The number of plants in a population declines each generation due to failure of some seeds to germinate or some plants to produce at least one seed. As a result, not all of the F_2 plants originally sampled in the population will be represented by a progeny when generation advance is completed.

Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several reference books (e.g., "Principles of Plant Breeding" John Wiley and Son, pp. 115-161, 1960; Allard, 1960; Simmonds, 1979; Sneep et al., 1979; Fehr, 1987).

Proper testing should detect any major faults and establish the level of superiority or improvement over current cultivars. In addition to showing superior performance, there must be a demand for a new cultivar that is compatible with industry standards or which creates a new market. The introduction of a new cultivar will incur additional costs to the seed producer, the grower, processor and consumer for special advertising and marketing, altered seed and commercial production practices, and new product utilization. The testing preceding release of a new cultivar should take into consideration research and development costs as well as technical superiority of the final cultivar. For seed-propagated cultivars, it must be feasible to produce seed easily and economically.

Lettuce in general and romaine lettuce in particular is an important and valuable vegetable crop. Thus, a continuing goal of lettuce plant breeders is to develop stable, high yielding lettuce cultivars that are agronomically sound. To accomplish this goal, the lettuce breeder must select and develop lettuce plants with traits that result in superior cultivars.

The foregoing examples of the related art and limitations related therewith are intended to be illustrative and not exclusive. Other limitations of the related art will become apparent to those of skill in the art upon a reading of the specification.

SUMMARY OF THE INVENTION

The following embodiments and aspects thereof are described and illustrated in conjunction with systems, tools and methods which are meant to be exemplary and illustrative, not limiting in scope. In various embodiments, one or more of the above-described problems have been reduced or eliminated, while other embodiments are directed to other improvements.

According to the invention, there is provided a novel romaine lettuce cultivar designated Green Thunder. This invention thus relates to the seeds of lettuce cultivar Green Thunder, to the plants of lettuce cultivar Green Thunder and to methods for producing a lettuce plant produced by crossing the lettuce Green Thunder with itself or another lettuce cultivar, and to methods for producing a lettuce plant containing in its genetic material one or more transgenes and to the transgenic lettuce plants produced by that method. This invention also relates to methods for producing other lettuce cultivars derived from lettuce cultivar Green Thunder and to the lettuce cultivar derived by the use of those methods. This invention further relates to hybrid lettuce seeds and plants produced by crossing the cultivar Green Thunder with another lettuce cultivar.

In another aspect, the present invention provides regenerable cells for use in tissue culture of lettuce cultivar Green Thunder. The tissue culture will preferably be capable of regenerating plants having the physiological and morphological characteristics of the foregoing lettuce plant, and of regenerating plants having substantially the same genotype as the foregoing lettuce plant. Preferably, the regenerable cells in such tissue cultures will be embryos, protoplasts, seeds, callus, pollen, leaves, anthers, pistils, roots, root tips and meristematic cells. Still further, the present invention provides lettuce plants regenerated from the tissue cultures of the invention.

Another aspect of the invention is to provide methods for producing other lettuce plants derived from lettuce cultivar Green Thunder. Lettuce cultivars derived by the use of those methods are also part of the invention.

The invention also relates to methods for producing a lettuce plant containing in its genetic material one or more transgenes and to the transgenic lettuce plant produced by that method.

In another aspect, the present invention provides for single gene converted plants of Green Thunder. The single transferred gene may preferably be a dominant or recessive allele. Preferably, the single transferred gene will confer such traits as male sterility, herbicide resistance, insect resistance, resistance for bacterial, fungal, or viral disease, male fertility, enhanced nutritional quality and industrial usage. The single gene may be a naturally occurring lettuce gene or a transgene introduced through genetic engineering techniques.

The invention further provides methods for developing lettuce plants in a lettuce plant breeding program using plant breeding techniques including recurrent selection, backcrossing, pedigree breeding, restriction fragment length polymorphism enhanced selection, genetic marker enhanced selection and transformation. Seeds, lettuce plants, and parts thereof produced by such breeding methods are also part of the invention.

In addition to the exemplary aspects and embodiments described above, further aspects and embodiments will become apparent by study of the following descriptions.

DEFINITIONS

In the description and tables which follow, a number of terms are used. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided:

Allele. The allele is any of one or more alternative form of a gene, all of which alleles relate to one trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

Backcrossing. Backcrossing is a process in which a breeder repeatedly crosses hybrid progeny back to one of the parents, for example, a first generation hybrid F_1 with one of the parental genotype of the F_1 hybrid.

Essentially all the physiological and morphological characteristics. A plant having essentially all the physiological and morphological characteristics means a plant having essentially all of the physiological and morphological characteristics of the recurrent parent, except for the characteristics derived from the converted gene.

Regeneration. Regeneration refers to the development of a plant from tissue culture.

Single gene converted. Single gene converted or conversion plant refers to plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological charac-

teristics of a cultivar are recovered in addition to the single gene transferred into the cultivar via the backcrossing technique or via genetic engineering.

Maturity Date. Maturity refers to the stage when the plants are of full size or optimum weight, in marketable form or shape to be of commercial or economic value. In romaine types they range from 50-75 days from time of seeding, depending upon the season of the year.

RHS. RHS refers to the Royal Horticultural Society of England which publishes an official botanical color chart quantitatively identifying colors according to a defined numbering system. The chart may be purchased from Royal Horticulture Society Enterprise Ltd RHS Garden; Wisley, Woking; Surrey GU236QB, UK.

Yield (Tons/Acre). The yield in tons/acre is the actual yield of the lettuce at harvest.

DETAILED DESCRIPTION OF THE INVENTION

Lettuce cultivar Green Thunder has superior characteristics and was developed from the cross 00BS-0719, a proprietary romaine breeding line, and Green Forest, which was made in the summer of 2000 in the greenhouse at Synergene Seed in California. The F_1 hybrids were grown in a greenhouse during the fall and winter of 2000 and 2001. F_2 selection was made at Synergene Seed Spring Nursery in the spring of 2001. The F_3 selections were made in the spring of 2002 at Synergene Seed Spring Nursery. F_4 plants were selected in 2003 in the Synergene Seed Spring Nursery. F_4 plants were selected and bulked in field plots in San Joaquin Valley, Calif. during the summers of 2003 and 2004.

Green Thunder is a romaine lettuce with a very dark green leaf color, thick and slightly blistered leaf texture, highly dense and V-shaped head; it has a very vigorous growth habit and is widely adoptable in a variety of environments. Green Thunder is resistant to tipburn and *Sclerotinia*. It is also highly tolerant to twisting and mid-rib deformity. Green Thunder has shown a very good adaptability in the coastal California and desert Arizona regions of the United States.

Some of the criteria used for selection in various generations include: color, disease resistance, head weight, number of leaves, appearance and length, yield, emergence, maturity, plant architecture, seed yield and quality.

The cultivar has shown uniformity and stability for the traits, within the limits of environmental influence for the traits. It has been self-pollinated a sufficient number of generations with careful attention to uniformity of plant type. The cultivar has been increased with continued observation for uniformity. No variant traits have been observed or are expected in Green Thunder.

Lettuce cultivar Green Thunder has the following morphologic and other characteristics (based primarily on data collected at Salinas, Calif.).

TABLE 1

VARIETY DESCRIPTION INFORMATION

Plant Type

Romaine
Seed

Color: Black
Light dormancy: Light not required
Heat dormancy: Susceptible
Cotyledon to Fourth Leaf Stage

Shape of cotyledons: Very Broad
Undulation: Flat

TABLE 1-continued

VARIETY DESCRIPTION INFORMATION	
Anthocyanin distribution: Absent	5
Rolling: Absent	
Cupping: Uncupped	
Reflexing: None	
<u>Mature Leaves</u>	
Margin - Incision depth: Absent/Shallow	
Margin - Indentation: Entire	
Margin - Undulation of the apical margin: Absent/Slightly	10
Green color: Very dark green	
Anthocyanin - Distribution: Absent	
Size: Large	
Glossiness: Glossy	
Blistering: Moderate	
Trichomes: Absent	
Leaf thickness: Thick	
<u>Plant at Market Stage</u>	
Head shape: Non-heading, V-shaped	
Head size class: Large	20
Head weight: 940 g	
Head firmness: Loose	
<u>Core</u>	
Diameter at base of head: 4.3 cm	25
Core height from base of head to apex: 6.7 cm	
<u>Maturity</u>	
Summer: 52 days	
Winter: 92 days	
<u>Adaptation</u>	
Primary Regions of Adaptation (tested and proven adapted)	
Southwest (California, Arizona desert): Adapted	
West Coast: Adapted	
Southeast: N/A	
Northeast: Adapted	35
Spring area: San Joaquin, Imperial, CA; Yuma, AZ	
Summer area: Salinas, Santa Maria, San Juan Bautista, CA	
Fall area: Salinas, Santa Maria, Oxnard, CA	
Winter area: Yuma, AZ; Imperial, Coachella, CA	
Greenhouse: N/A	
Soil Type: Both Mineral and Organic	40
Disease and Stress Reactions	
<u>Virus</u>	
Big Vein: Intermediate	
Lettuce Mosaic: Susceptible	45
Cucumber Mosaic: Not tested	
Broad Bean Wilt: Not tested	
Turnip Mosaic: Not tested	
Best Western Yellows: Not tested	
Lettuce Infectious Yellows: Not tested	
<u>Fungal/Bacterial</u>	
Corky Root Rot (<i>Pythium</i> Root Rot): Intermediate	50
Downy Mildew: Susceptible	
Powdery Mildew: Not tested	
<i>Sclerotinia</i> Rot: Highly resistant	
Bacterial Soft Rot (<i>Pseudomonas</i> sp. & others): Not tested	
<i>Botrytis</i> (Gray Mold): Susceptible	55
<u>Insects</u>	
Cabbage Loopers: Susceptible	
Root Aphids: Susceptible	60
Green Peach Aphid: Susceptible	
<u>Physiological/Stress</u>	
Tipburn: Highly resistant	
Heat: Intermediate	
Drought: Not tested	
Cold: Resistant	
Salt: Not tested	65
Brown Rib: Resistant	

TABLE 1-continued

VARIETY DESCRIPTION INFORMATION	
	5
<u>Post Harvest</u>	
Pink Rib: Resistant	
Russet Spotting: Not tested	
Rusty Brown Discoloration: Not tested	
Internal Rib Necrosis (Blackheart, Gray Rib, Gray Streak): Resistant	10
Brown Stain: Not tested	

FURTHER EMBODIMENTS OF THE INVENTION

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This invention also is directed to methods for producing a lettuce cultivar plant by crossing a first parent lettuce plant with a second parent lettuce plant wherein either the first or second parent lettuce plant is a lettuce plant of the cultivar Green Thunder. Further, both first and second parent lettuce plants can come from the cultivar Green Thunder. Still further, this invention also is directed to methods for producing a cultivar Green Thunder-derived lettuce plant by crossing cultivar Green Thunder with a second lettuce plant and growing the progeny seed, and repeating the crossing and growing steps with the cultivar Green Thunder-derived plant from 0 to 7 times. Thus, any such methods using the cultivar Green Thunder are part of this invention: selfing, backcrosses, hybrid production, crosses to populations, and the like. All plants produced using cultivar Green Thunder as a parent are within the scope of this invention, including plants derived from cultivar Green Thunder. Advantageously, the cultivar is used in crosses with other, different, cultivars to produce first generation (F₁) lettuce seeds and plants with superior characteristics.

As used herein, the term plant includes plant cells, plant protoplasts, plant cell tissue cultures from which lettuce plants can be regenerated, plant calli, plant clumps and plant cells that are intact in plants or parts of plants, such as embryos, pollen, ovules, flowers, seeds, roots, anthers, pistils and the like.

As is well known in the art, tissue culture of lettuce can be used for the in vitro regeneration of a lettuce plant. Tissue culture of various tissues of lettuces and regeneration of plants therefrom is well known and widely published. For example, reference may be had to Teng et al., *HortScience*. 1992, 27: 9, 1030-1032, Teng et al., *HortScience*. 1993, 28: 6, 669-1671, Zhang et al., *Journal of Genetics and Breeding*. 1992, 46: 3, 287-290, Webb et al., *Plant Cell Tissue and Organ Culture*. 1994, 38: 1, 77-79, Curtis et al., *Journal of Experimental Botany*. 1994, 45: 279, 1441-1449, Nagata et al., *Journal for the American Society for Horticultural Science*. 2000, 125: 6, 669-672. It is clear from the literature that the state of the art is such that these methods of obtaining plants are "conventional" in the sense that they are routinely used and have a very high rate of success. Thus, another aspect of this invention is to provide cells which upon growth and differentiation produce lettuce plants having the physiological and morphological characteristics of variety Green Thunder.

With the advent of molecular biological techniques that have allowed the isolation and characterization of genes that encode specific protein products, scientists in the field of plant biology developed a strong interest in engineering the genomes of plants to contain and express foreign genes, or additional, or modified versions of native, or endogenous,

genes (perhaps driven by different promoters) in order to alter the traits of a plant in a specific manner. Such foreign additional and/or modified genes are referred to herein collectively as “transgenes”. Over the last fifteen to twenty years several methods for producing transgenic plants have been developed, and the present invention in particular embodies also relates to transformed versions of the claimed cultivar.

Plant transformation involves the construction of an expression vector that will function in plant cells. Such a vector comprises DNA comprising a gene under control of, or operatively linked to, a regulatory element (for example, a promoter). The expression vector may contain one or more such operably linked gene/regulatory element combinations. The vector(s) may be in the form of a plasmid, and can be used alone or in combination with other plasmids, to provide transformed lettuce plants, using transformation methods as described below to incorporate transgenes into the genetic material of the lettuce plant(s).

Expression Vectors for Lettuce Transformation-Markers

Expression vectors include at least one genetic marker, operably linked to a regulatory element (a promoter, for example) that allows transformed cells containing the marker to be either recovered by negative selection, i.e., inhibiting growth of cells that do not contain the selectable marker gene, or by positive selection, i.e., screening for the product encoded by the genetic marker. Many commonly used selectable marker genes for plant transformation are well known in the transformation arts, and include, for example, genes that code for enzymes that metabolically detoxify a selective chemical agent which may be an antibiotic or an herbicide, or genes that encode an altered target which is insensitive to the inhibitor. A few positive selection methods are also known in the art.

One commonly used selectable marker gene for plant transformation is the neomycin phosphotransferase II (nptII) gene, isolated from transposon Tn5, which when placed under the control of plant regulatory signals confers resistance to kanamycin. Fraley et al., *Proc. Natl. Acad. Sci. U.S.A.*, 80:4803 (1983). Another commonly used selectable marker gene is the hygromycin phosphotransferase gene which confers resistance to the antibiotic hygromycin. Vanden Elzen et al., *Plant Mol. Biol.*, 5:299 (1985).

Additional selectable marker genes of bacterial origin that confer resistance to antibiotics include gentamycin acetyl transferase, streptomycin phosphotransferase and aminoglycoside-3'-adenyl transferase, the bleomycin resistance determinant. Hayford et al., *Plant Physiol.* 86:1216 (1988), Jones et al., *Mol. Gen. Genet.*, 210:86 (1987), Svab et al., *Plant Mol. Biol.* 14:197 (1990) Hille et al., *Plant Mol. Biol.* 7:171 (1986). Other selectable marker genes confer resistance to herbicides such as glyphosate, glufosinate or broxynil. Comai et al., *Nature* 317:741-744 (1985), Gordon-Kamm et al., *Plant Cell* 2:603-618 (1990) and Stalker et al., *Science* 242:419-423 (1988).

Selectable marker genes for plant transformation that are not of bacterial origin include, for example, mouse dihydrofolate reductase, plant 5-enolpyruvylshikimate-3-phosphate synthase and plant acetolactate synthase. Eichholtz et al., *Somatic Cell Mol. Genet.* 13:67 (1987), Shah et al., *Science* 233:478 (1986), Charest et al., *Plant Cell Rep.* 8:643 (1990).

Another class of marker genes for plant transformation requires screening of presumptively transformed plant cells rather than direct genetic selection of transformed cells for resistance to a toxic substance such as an antibiotic. These

genes are particularly useful to quantify or visualize the spatial pattern of expression of a gene in specific tissues and are frequently referred to as reporter genes because they can be fused to a gene or gene regulatory sequence for the investigation of gene expression. Commonly used genes for screening presumptively transformed cells include β -glucuronidase (GUS), α -galactosidase, luciferase and chloramphenicol acetyltransferase. Jefferson, R. A., *Plant Mol. Biol. Rep.* 5:387 (1987), Teeri et al., *EMBO J.* 8:343 (1989), Koncz et al., *Proc. Natl. Acad. Sci U.S.A.* 84:131 (1987), DeBlock et al., *EMBO J.* 3:1681 (1984).

In vivo methods for visualizing GUS activity that do not require destruction of plant tissue are available. Molecular Probes publication 2908, Imagen Green, p. 1-4 (1993) and Naleway et al., *J. Cell Biol.* 115:151a (1991). However, these in vivo methods for visualizing GUS activity have not proven useful for recovery of transformed cells because of low sensitivity, high fluorescent backgrounds and limitations associated with the use of luciferase genes as selectable markers.

More recently, a gene encoding Green Fluorescent Protein (GFP) has been utilized as a marker for gene expression in prokaryotic and eukaryotic cells. Chalfie et al., *Science* 263:802 (1994). GFP and mutants of GFP may be used as screenable markers.

Expression Vectors for Lettuce Transformation-Promoters

Genes included in expression vectors must be driven by a nucleotide sequence comprising a regulatory element, for example, a promoter. Several types of promoters are well known in the transformation arts, as are other regulatory elements that can be used alone or in combination with promoters.

As used herein, “promoter” includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A “plant promoter” is a promoter capable of initiating transcription in plant cells. Examples of promoters under developmental control include promoters that preferentially initiate transcription in certain tissues, such as leaves, roots, seeds, fibers, xylem vessels, tracheids, or sclerenchyma. Such promoters are referred to as “tissue-preferred”. Promoters which initiate transcription only in certain tissue are referred to as “tissue-specific”. A “cell type” specific promoter primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots or leaves. An “inducible” promoter is a promoter which is under environmental control. Examples of environmental conditions that may effect transcription by inducible promoters include anaerobic conditions or the presence of light. Tissue-specific, tissue-preferred, cell type specific, and inducible promoters constitute the class of “non-constitutive” promoters. A “constitutive” promoter is a promoter which is active under most environmental conditions.

A. Inducible Promoters

An inducible promoter is operably linked to a gene for expression in lettuce. Optionally, the inducible promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a gene for expression in lettuce. With an inducible promoter the rate of transcription increases in response to an inducing agent.

Any inducible promoter can be used in the instant invention. See Ward et al., *Plant Mol. Biol.* 22:361-366 (1993). Exemplary inducible promoters include, but are not limited to, that from the ACEI system which responds to copper (Meft et al., *PNAS* 90:4567-4571 (1993)); In2 gene from

maize which responds to benzenesulfonamide herbicide safeners (Hershey et al., *Mol. Gen. Genetics* 227:229-237 (1991) and Gatz et al., *Mol. Gen. Genetics* 243:32-38 (1994)) or Tet repressor from Tn10 (Gatz et al., *Mol. Gen. Genetics* 227:229-237 (1991)). A particularly preferred inducible promoter is a promoter that responds to an inducing agent to which plants do not normally respond. An exemplary inducible promoter is the inducible promoter from a steroid hormone gene, the transcriptional activity of which is induced by a glucocorticosteroid hormone. Schena et al., *Proc. Natl. Acad. Sci. U.S.A.* 88:0421 (1991).

B. Constitutive Promoters

A constitutive promoter is operably linked to a gene for expression in lettuce or the constitutive promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a gene for expression in lettuce.

Many different constitutive promoters can be utilized in the instant invention. Exemplary constitutive promoters include, but are not limited to, the promoters from plant viruses such as the 35S promoter from CaMV (Odell et al., *Nature* 313:810-812 (1985) and the promoters from such genes as rice actin (McElroy et al., *Plant Cell* 2:163-171 (1990)); ubiquitin (Christensen et al., *Plant Mol. Biol.* 12:619-632 (1989) and Christensen et al., *Plant Mol. Biol.* 18:675-689 (1992)); pEMU (Last et al., *Theor. Appl. Genet.* 81:581-588 (1991)); MAS (Velten et al., *EMBO J.* 3:2723-2730 (1984)) and maize H3 histone (Lepetit et al., *Mol. Gen. Genetics* 231:276-285 (1992) and Atanassova et al., *Plant Journal* 2 (3): 291-300 (1992)).

The ALS promoter, Xba1/NcoI fragment 5' to the *Brassica napus* ALS3 structural gene (or a nucleotide sequence similarity to said Xba1/NcoI fragment), represents a particularly useful constitutive promoter. See PCT application WO96/30530.

C. Tissue-Specific or Tissue-Preferred Promoters

A tissue-specific promoter is operably linked to a gene for expression in lettuce. Optionally, the tissue-specific promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a gene for expression in lettuce. Plants transformed with a gene of interest operably linked to a tissue-specific promoter produce the protein product of the transgene exclusively, or preferentially, in a specific tissue.

Any tissue-specific or tissue-preferred promoter can be utilized in the instant invention. Exemplary tissue-specific or tissue-preferred promoters include, but are not limited to, a root-preferred promoter, such as that from the phaseolin gene (Murai et al., *Science* 23:476-482 (1983) and Sengupta-Gopalan et al., *Proc. Natl. Acad. Sci. U.S.A.* 82:3320-3324 (1985)); a leaf-specific and light-induced promoter such as that from cab or rubisco (Simpson et al., *EMBO J.* 4(11):2723-2729 (1985) and Timko et al., *Nature* 318:579-582 (1985)); an anther-specific promoter such as that from LAT52 (Twell et al., *Mol. Gen. Genetics* 217:240-245 (1989)); a pollen-specific promoter such as that from Zm13 (Guerrero et al., *Mol. Gen. Genetics* 244:161-168 (1993)) or a microspore-preferred promoter such as that from apg (Twell et al., *Sex. Plant Reprod.* 6:217-224 (1993)).

D. Signal Sequences for Targeting Proteins to Subcellular Compartments

Transport of protein produced by transgenes to a subcellular compartment such as the chloroplast, vacuole, peroxisome, glyoxysome, cell wall or mitochondrion or for secretion into the apoplast, is accomplished by means of operably linking the nucleotide sequence encoding a signal sequence to the 5' and/or 3' region of a gene encoding the protein of interest. Targeting sequences at the 5' and/or 3' end of the

structural gene may determine, during protein synthesis and processing, where the encoded protein is ultimately compartmentalized.

The presence of a signal sequence directs a polypeptide to either an intracellular organelle or subcellular compartment or for secretion to the apoplast. Many signal sequences are known in the art. See, for example Becker et al., *Plant Mol. Biol.* 20:49 (1992), Knox, C., et al., *Plant Mol. Biol.* 9:3-17 (1987), Lerner et al., *Plant Physiol.* 91:124-129 (1989), Fontes et al., *Plant Cell* 3:483-496 (1991), Matsuoka et al., *Proc. Natl. Acad. Sci.* 88:834 (1991), Gould et al., *J. Cell. Biol.* 108:1657 (1989), Creissen et al., *Plant J.* 2:129 (1991), Kalderon, et al., *Cell* 39:499-509 (1984), and Steifel, et al., *Plant Cell* 2:785-793 (1990).

E. Foreign Protein Genes and Agronomic Genes

With transgenic plants according to the present invention, a foreign protein can be produced in commercial quantities. Thus, techniques for the selection and propagation of transformed plants, which are well understood in the art, yield a plurality of transgenic plants which are harvested in a conventional manner, and a foreign protein then can be extracted from a tissue of interest or from total biomass. Protein extraction from plant biomass can be accomplished by known methods which are discussed, for example, by Heney and Orr, *Anal. Biochem.* 114:92-6 (1981).

According to a preferred embodiment, the transgenic plant provided for commercial production of foreign protein is lettuce. In another preferred embodiment, the biomass of interest is seed. For the relatively small number of transgenic plants that show higher levels of expression, a genetic map can be generated, primarily via conventional RFLP, PCR and SSR analysis, which identifies the approximate chromosomal location of the integrated DNA molecule. For exemplary methodologies in this regard, see Glick and Thompson, *Methods in Plant Molecular Biology and Biotechnology CRC Press, Boca Raton* 269:284 (1993). Map information concerning chromosomal location is useful for proprietary protection of a subject transgenic plant. If unauthorized propagation is undertaken and crosses made with other germplasm, the map of the integration region can be compared to similar maps for suspect plants, to determine if the latter have a common parentage with the subject plant. Map comparisons would involve hybridizations, RFLP, PCR, SSR and sequencing, all of which are conventional techniques.

Likewise, by means of the present invention, agronomic genes can be expressed in transformed plants. More particularly, plants can be genetically engineered to express various phenotypes of agronomic interest. Exemplary genes implicated in this regard include, but are not limited to, those categorized below:

1. Genes that Confer Resistance to Pests or Disease and that Encode

A. Plant disease resistance genes. Plant defenses are often activated by specific interaction between the product of a disease resistance gene (R) in the plant and the product of a corresponding avirulence (Avr) gene in the pathogen. A plant cultivar can be transformed with a cloned resistance gene(s) to engineer plants that are resistant to specific pathogen strains. See, for example Jones et al., *Science* 266:789 (1994) (cloning of the tomato Cf-9 gene for resistance to *Cladosporium fulvum*); Martin et al., *Science* 262:1432 (1993) (tomato Pto gene for resistance to *Pseudomonas syringae* pv. tomato encodes a protein kinase); Mindrinos et al., *Cell* 78:1089 (1994) (*Arabidopsis* RSP2 gene for resistance to *Pseudomonas syringae*).

B. A *Bacillus thuringiensis* protein, a derivative thereof or a synthetic polypeptide modeled thereon. See, for example, Geiser et al., *Gene* 48:109 (1986), who disclose the cloning and nucleotide sequence of a Bt α -endotoxin gene. Moreover, DNA molecules encoding α -endotoxin genes can be

purchased from American Type Culture Collection, Manassas, Va., for example, under ATCC Accession Nos. 40098, 67136, 31995 and 31998.

C. A lectin. See, for example, the disclosure by Van Damme et al., *Plant Molec. Biol.* 24:25 (1994), who disclose

the nucleotide sequences of several *Clivia miniata* mannose-binding lectin genes.

D. A vitamin-binding protein such as avidin. See PCT application US93/06487. The application teaches the use of avidin and avidin homologues as larvicides against insect

pests.

E. An enzyme inhibitor, for example, a protease or proteinase inhibitor or an amylase inhibitor. See, for example, Abe et al., *J. Biol. Chem.* 262:16793 (1987) (nucleotide sequence of rice cysteine proteinase inhibitor), Huub et al., *Plant Molec. Biol.* 21:985 (1993) (nucleotide sequence of cDNA encoding tobacco proteinase inhibitor I), Sumitani et al., *Biosci. Biotech. Biochem.* 57: 1243 (1993) (nucleotide sequence of *Streptomyces nitrosporeus* α -amylase inhibitor).

F. An insect-specific hormone or pheromone such as an ecdysteroid or juvenile hormone, a variant thereof, a mimetic based thereon, or an antagonist or agonist thereof. See, for example, the disclosure by Hammock et al., *Nature* 344:458 (1990), of baculovirus expression of cloned juvenile hormone esterase, an inactivator of juvenile hormone.

G. An insect-specific peptide or neuropeptide which, upon expression, disrupts the physiology of the affected pest. For example, see the disclosures of Regan, *J. Biol. Chem.* 269:9 (1994) (expression cloning yields DNA coding for insect diuretic hormone receptor), and Pratt et al., *Biochem. Biophys. Res. Comm.* 163:1243 (1989) (an allostatin is identified in *Diploptera puntata*). See also U.S. Pat. No. 5,266,317 to Tomalski et al., who disclose genes encoding insect-specific, paralytic neurotoxins.

H. An insect-specific venom produced in nature by a snake, a wasp, etc. For example, see Pang et al., *Gene* 116:165 (1992), for disclosure of heterologous expression in plants of a gene coding for a scorpion insectotoxic peptide.

I. An enzyme responsible for a hyper-accumulation of a monoterpene, a sesquiterpene, a steroid, a hydroxamic acid, a phenylpropanoid derivative or another non-protein molecule with insecticidal activity.

J. An enzyme involved in the modification, including the post-translational modification, of a biologically active molecule; for example, a glycolytic enzyme, a proteolytic enzyme, a lipolytic enzyme, a nuclease, a cyclase, a transaminase, an esterase, a hydrolase, a phosphatase, a kinase, a phosphorylase, a polymerase, an elastase, a chitinase and a glucanase, whether natural or synthetic. See PCT application WO 93/02197 in the name of Scott et al., which discloses the nucleotide sequence of a callase gene. DNA molecules which contain chitinase-encoding sequences can be obtained, for example, from the ATCC under Accession Nos. 39637 and 67152. See also Kramer et al., *Insect Biochem. Molec. Biol.* 23:691 (1993), who teach the nucleotide sequence of a cDNA encoding tobacco hookworm chitinase, and Kawalleck et al., *Plant Molec. Biol.* 21:673 (1993), who provide the nucleotide sequence of the parsley ubi4-2 polyubiquitin gene.

K. A molecule that stimulates signal transduction. For example, see the disclosure by Botella et al., *Plant Molec.*

Biol. 24:757 (1994), of nucleotide sequences for mung lettuce calmodulin cDNA clones, and Griess et al., *Plant Physiol.* 104:1467 (1994), who provide the nucleotide sequence of a maize calmodulin cDNA clone.

L. A hydrophobic moment peptide. See PCT application WO 95/16776 (disclosure of peptide derivatives of Tachyplesin which inhibit fungal plant pathogens) and PCT application WO 95/18855 (teaches synthetic antimicrobial peptides that confer disease resistance).

M. A membrane permease, a channel former or a channel blocker. For example, see the disclosure of Jaynes et al., *Plant Sci* 89:43 (1993), of heterologous expression of a cecropin- α , lytic peptide analog to render transgenic tobacco plants resistant to *Pseudomonas solanacearum*.

N. A viral-invasive protein or a complex toxin derived therefrom. For example, the accumulation of viral coat proteins in transformed plant cells imparts resistance to viral infection and/or disease development effected by the virus from which the coat protein gene is derived, as well as by related viruses. See Beachy et al., *Ann. Rev. Phytopathol.* 28: 451 (1990). Coat protein-mediated resistance has been conferred upon transformed plants against alfalfa mosaic virus, cucumber mosaic virus, tobacco streak virus, potato virus X, potato virus Y, tobacco etch virus, tobacco rattle virus and tobacco mosaic virus. Id.

O. An insect-specific antibody or an immunotoxin derived therefrom. Thus, an antibody targeted to a critical metabolic function in the insect gut would inactivate an affected enzyme, killing the insect. Cf. Taylor et al., Abstract #497, Seventh Int'l Symposium on Molecular Plant-Microbe Interactions (Edinburgh, Scotland) (1994) (enzymatic inactivation in transgenic tobacco via production of single-chain antibody fragments).

P. A virus-specific antibody. See, for example, Tavladoraki et al., *Nature* 366:469 (1993), who show that transgenic plants expressing recombinant antibody genes are protected from virus attack.

Q. A developmental-arrestive protein produced in nature by a pathogen or a parasite. Thus, fungal endo α -1,4-D-polygalacturonases facilitate fungal colonization and plant nutrient release by solubilizing plant cell wall homo- α -1,4-D-galacturonase. See Lamb et al., *Bio/Technology* 10:1436 (1992). The cloning and characterization of a gene which encodes a lettuce endopolygalacturonase-inhibiting protein is described by Toubart et al., *Plant J.* 2:367 (1992).

R. A developmental-arrestive protein produced in nature by a plant. For example, Logemann et al., *Bio/Technology* 10:305 (1992), have shown that transgenic plants expressing the barley ribosome-inactivating gene have an increased resistance to fungal disease.

S. A lettuce mosaic potyvirus (LMV) coat protein gene introduced into *Lactuca sativa* in order to increase its resistance to LMV infection. See Dinant et al., *Molecular Breeding*. 1997, 3:175-86.

2. Genes that Confer Resistance to an Herbicide, for Example:

A. An herbicide that inhibits the growing point or meristem, such as an imidazalinone or a sulfonyleurea. Exemplary genes in this category code for mutant ALS and AHAS enzymes as described, for example, by Lee et al., *EMBO J.* 7:1241 (1988), and Miki et al., *Theor. Appl. Genet.* 80:449 (1990), respectively.

B. Glyphosate (resistance impaired by mutant 5-enolpyruvyl-3-phosphoshikimate synthase (EPSP) and aroA genes, respectively) and other phosphono compounds such as glufosinate (phosphinothricin acetyl transferase (PAT) and *Streptomyces hygroscopicus* PAT, bar, genes), and

pyridinoxy or phenoxy propionic acids and cyclohexones (ACCase inhibitor-encoding genes). See, for example, U.S. Pat. No. 4,940,835 to Shah, et al., which discloses the nucleotide sequence of a form of EPSP which can confer glyphosate resistance. A DNA molecule encoding a mutant aroA gene can be obtained under ATCC accession number 39256, and the nucleotide sequence of the mutant gene is disclosed in U.S. Pat. No. 4,769,061 to Comai. See also Umaballava-Mobapathie in *Transgenic Research*. 1999, 8:1, 33-44, that discloses *Lactuca sativa* resistant to glufosinate. European patent application No. 0 333 033 to Kumada et al., and U.S. Pat. No. 4,975,374 to Goodman et al., disclose nucleotide sequences of glutamine synthetase genes which confer resistance to herbicides such as L-phosphinothricin. The nucleotide sequence of a phosphinothricin-acetyl-transferase gene is provided in European application No. 0 242 246 to Leemans et al.; DeGreef et al., *Bio/Technology* 7:61 (1989), describe the production of transgenic plants that express chimeric bar genes coding for phosphinothricin acetyl transferase activity. Examples of genes conferring resistance to phenoxy propionic acids and cyclohexones, such as sethoxydim and haloxyfop are the Acc1-S1, Acc1-S2 and Acc1-S3 genes are described by Marshall et al., *Theor. Appl. Genet.* 83:435 (1992).

C. An herbicide that inhibits photosynthesis, such as a triazine (psbA and gs+ genes) and a benzonitrile (nitrilase gene). Przibilla et al., *Plant Cell* 3:169 (1991), describe the transformation of *Chlamydomonas* with plasmids encoding mutant psbA genes. Nucleotide sequences for nitrilase genes are disclosed in U.S. Pat. No. 4,810,648 to Stalker, and DNA molecules containing these genes are available under ATCC Accession Nos. 53435, 67441, and 67442. Cloning and expression of DNA coding for a glutathione S-transferase is described by Hayes et al., *Biochem. J.* 285:173 (1992).

3. Genes that Confer or Contribute to a Value-Added Trait, Such as:

A. Increased iron content of the lettuce, for example by transforming a plant with a soybean ferritin gene as described in Goto et al., *Acta Horticulturae*. 2000, 521, 101-109. Parallel to the improved iron content, enhanced growth of transgenic lettuces was also observed in early development stages.

B. Decreased nitrate content of leaves, for example by transforming a lettuce with a gene coding for a nitrate reductase. See for example Curtis et al., *Plant Cell Report*. 1999, 18: 11, 889-896.

C. Increased sweetness of the lettuce by transferring a gene coding for monellin that elicits a flavor 100,000 times sweeter than sugar on a molar basis. See Penarrubia et al., *Biotechnology*. 1992, 10: 5, 561-564.

Numerous methods for plant transformation have been developed, including biological and physical plant transformation protocols. See, for example, Miki et al., "Procedures for Introducing Foreign DNA into Plants" in *Methods in Plant Molecular Biology and Biotechnology*, Glick B. R. and Thompson, J. E. Eds., CRC Press, Inc., Boca Raton, (1993) pages 67-88. In addition, expression vectors and in vitro culture methods for plant cell or tissue transformation and regeneration of plants are available. See, for example, Gruber et al., "Vectors for Plant Transformation" in *Methods in Plant Molecular Biology and Biotechnology*, Glick B. R. and Thompson, J. E. Eds., CRC Press, Inc., Boca Raton 1993 pages 89-119.

A. *Agrobacterium*-Mediated Transformation

One method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium*. See, for example, Horsch et al., *Science*

227:1229 (1985). Curtis et al., *Journal of Experimental Botany*. 1994, 45: 279, 1441-1449, Torres et al., *Plant Cell Tissue and Organic Culture*. 1993, 34: 3, 279-285, Dinant et al., *Molecular Breeding*. 1997, 3: 1, 75-86. *A. tumefaciens* and *A. rhizogenes* are plant pathogenic soil bacteria which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectively, carry genes responsible for genetic transformation of the plant. See, for example, Kado, C. I., *Crit. Rev. Plant Sci.* 10:1 (1991). Descriptions of *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided by Gruber et al., supra, Miki et al., supra, and Moloney et al., *Plant Cell Reports* 8:238 (1989). See also, U.S. Pat. No. 5,591,616 issued Jan. 7, 1997.

B. Direct Gene Transfer

Despite the fact the host range for *Agrobacterium*-mediated transformation is broad, some major cereal or vegetable crop species and gymnosperms have generally been recalcitrant to this mode of gene transfer, even though some success has recently been achieved in rice and corn. Hiei et al., *The Plant Journal* 6:271-282 (1994) and U.S. Pat. No. 5,591,616 issued Jan. 7, 1997. Several methods of plant transformation, collectively referred to as direct gene transfer, have been developed as an alternative to *Agrobacterium*-mediated transformation.

A generally applicable method of plant transformation is microprojectile-mediated transformation wherein DNA is carried on the surface of microprojectiles measuring 1 to 4 μm . The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate plant cell walls and membranes. Russell, D. R., et al. *Pl. Cell. Rep.* 12 (3 January), 165-169 (1993); Aragao, F. J. L., et al. *Plant Mol. Biol.* 20 (2 October); 357-359 (1992); Aragao, F. J. L., et al. *Pl. Cell. Rep.* 12 (9 July) 483-490 (1993); Aragao *Theor. Appl. Genet.* 93:142-150 (1996); Kim, J.; Minamikawa, T. *Plant Science* 117: 131-138 (1996); Sanford et al., *Part. Sci. Technol.* 5:27 (1987); Sanford, J. C., *Trends Biotech.* 6:299 (1988); Klein et al., *Bio/Technology* 6:559-563 (1988); Sanford, J. C., *Physiol Plant* 7:206 (1990); Klein et al., *Biotechnology* 10:268 (1992).

Another method for physical delivery of DNA to plants is sonication of target cells. Zhang et al., *Bio/Technology* 9:996 (1991). Alternatively, liposome and spheroplast fusion have been used to introduce expression vectors into plants. Deshayes et al., *EMBO J.*, 4:2731 (1985), Christou et al., *Proc Natl. Acad. Sci. U.S.A.* 84:3962 (1987). Direct uptake of DNA into protoplasts using CaCl_2 precipitation, polyvinyl alcohol or poly-L-ornithine has also been reported. Hain et al., *Mol. Gen. Genet.* 199:161 (1985) and Draper et al., *Plant Cell Physiol.* 23:451 (1982). Electroporation of protoplasts and whole cells and tissues has also been described. Saker, M.; Kuhne, T. *Biologia Plantarum* 40(4): 507-514 (1997/98), Donn et al., In Abstracts of VIIth International Congress on Plant Cell and Tissue Culture IAPTC, A2-38, p 53 (1990); D'Halluin et al., *Plant Cell* 4:1495-1505 (1992) and Spencer et al., *Plant Mol. Biol.* 24:51-61 (1994). See also Chupean et al., *Biotechnology*. 1989, 7: 5, 503-508.

Following transformation of lettuce target tissues, expression of the above-described selectable marker genes allows for preferential selection of transformed cells, tissues and/or plants, using regeneration and selection methods now well known in the art.

The foregoing methods for transformation would typically be used for producing a transgenic cultivar. The transgenic cultivar could then be crossed with another (non-transformed or transformed) cultivar in order to produce a new transgenic lettuce cultivar. Alternatively, a genetic trait which has been engineered into a particular lettuce cultivar using the foregoing transformation techniques could be moved into another cultivar using traditional backcrossing techniques that are well known in the plant breeding arts. For example, a backcrossing approach could be used to move an engineered trait from a public, non-elite inbred cultivar into an elite inbred cultivar, or from an inbred cultivar containing a foreign gene in its genome into an inbred cultivar or cultivars which do not contain that gene. As used herein, "crossing" can refer to a simple X by Y cross, or the process of backcrossing, depending on the context.

When the terms lettuce plant, cultivar or lettuce cultivar are used in the context of the present invention, this also includes any single gene conversions of that cultivar. The term single gene converted plant as used herein refers to those lettuce plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of a cultivar are recovered in addition to the single gene transferred into the cultivar via the backcrossing technique. Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the cultivar. The term backcrossing as used herein refers to the repeated crossing of a hybrid progeny back to one of the parental lettuce plants for that cultivar. The parental lettuce plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental lettuce plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol (Poehlman & Sleper, 1994; Fehr, 1987). In a typical backcross protocol, the original cultivar of interest (recurrent parent) is crossed to a second cultivar (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a lettuce plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the converted plant, in addition to the single transferred gene from the nonrecurrent parent.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original cultivar. To accomplish this, a single gene of the recurrent cultivar is modified or substituted with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genetic, and therefore the desired physiological and morphological, constitution of the original cultivar. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross; one of the major purposes is to add some commercially desirable and/or agronomically important trait to the plant. The exact backcrossing protocol will depend on the characteristic or trait being altered to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred.

Many single gene traits have been identified that are not regularly selected for in the development of a new cultivar but that can be improved by backcrossing techniques. Single gene traits may or may not be transgenic, examples of these traits include but are not limited to, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, enhanced nutritional quality, industrial usage, yield stability and yield enhancement. These genes are generally inherited through the nucleus. Several of these single gene traits are described in U.S. Pat. Nos. 5,777,196, 5,948,957 and 5,969,212, the disclosures of which are specifically hereby incorporated by reference.

TABLES

In the tables that follow, the traits and characteristics of lettuce cultivar Green Thunder are given compared to two commercial romaine lettuce cultivars, Green Forest and PIC Cos.

Table 2 below shows the mature seed stalk height for Green Thunder as compared to the seed stalk height for Green Forest and PIC Cos. Seed stalk height is measured in centimeters. An analysis of variance was performed on the data and is shown below the data. As can be seen from the data in Table 2, Green Thunder has a significantly taller mature seed stalk height than either Green Forest or PIC Cos.

TABLE 2

Seed Stalk Height (cm)		
Green Thunder	Green Forest	PIC Cos
105	101	100
107	105	102
109	102	100
108	100	95
100	98	96
102	105	105

TABLE 2-continued

103	103	97
111	99	98
110	96	99
101	97	98
102	100	99
100	106	100
110	95	96

Anova: Single Factor SUMMARY				
Groups	Count	Sum	Average	Variance
Green Thunder	13	1368	105.2307692	16.85897
Green Forest	13	1307	100.5384615	12.60256
PIC Cos	13	1285	98.84615385	7.307692

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	284.4615385	2	142.2307692	11.6046	0.000129	3.259444
Within Groups	441.2307692	36	12.25641026			
Total	725.6923077	38				

25

Table 3 below shows the seed stalk spread for Green Thunder as compared to the seed stalk spread for Green Forest and PIC Cos. Seed stalk spread is measured in centimeters. An analysis of variance was performed on the data and is shown below the data. As can be seen from the data in Table 3, Green Thunder has a significantly wider mature seed stalk spread than Green Forest but a narrower mature seed stalk spread than PIC Cos.

Tables 4 through 8 show data collected in eight different locations. Location 1 was near Bard, Calif., location 2 was near San Juan Bautista, Calif., locations 3, 5, 6, 7, and 8 were near Salinas, Calif. and location 4 was near Yuma, Ariz.

30 Table 4 below shows the plant weight in grams at harvest maturity of Green Thunder as compared to Green Forest and PIC Cos. An analysis of variance was performed on the data and is shown below the data. As can be seen from the data in Table 4, Green Thunder is significantly heavier in plant

TABLE 3

Seed Stalk Spread (cm)			
Green Thunder	Green Forest	PIC Cos	
37	44	52	
50	42	45	
44	42	47	
45	40	41	
45	40	48	
46	45	41	
38	44	42	
42	42	47	
43	35	43	
46	44	48	
40	36	44	
45	44	45	
44	40	40	

Anova: Single Factor SUMMARY				
Groups	Count	Sum	Average	Variance
Green Thunder	13	565	43.46153846	12.4359
Green Forest	13	538	41.38461538	9.75641
PIC Cos	13	583	44.84615385	12.14103

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	78.92307692	2	39.46153846	3.448096	0.042649	3.259444
Within Groups	412	36	11.44444444			
Total	490.9230769	38				

weight at harvest maturity than either Green Forest or PIC Cos.

TABLE 4

Trial	Plant Weight (g) at Harvest Maturity							
	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
Green Thunder	1020	908	1021	1201	1021	1201	540	805
	1160	1160	984	1136	984	1136	790	780
	936	1160	908	1165	908	1165	810	810
	908	1020	1025	1146	1025	1146	790	650
	908	681	984	990	984	990	810	820
	846	1076	1026	1025	1026	1025	570	870
	846	790	988	914	988	914	85	650
	1020	965	956	986	956	986	860	750
	1244	681	1024	1034	1024	1034	820	750
	1104	795	1136	1130	1136	1130	790	650
Green Forest	820	622	756	759	756	759	670	680
	795	1076	687	774	687	774	710	690
	1076	681	909	804	909	804	610	720
	874	795	675	907	675	907	580	820
	908	795	756	682	756	682	690	750
	820	1076	764	673	764	673	620	800
	908	622	904	698	904	698	710	720
	1244	795	689	768	6889	768	550	780
	681	681	677	759	677	759	690	820
	846	846	757	683	757	683	710	810
PIC Cos	846	760	688	766	688	766	610	650
	965	820	901	771	901	771	710	720
	875	795	679	699	679	699	540	590
	705	622	766	706	766	706	600	680
	846	740	752	755	752	755	580	710
	965	622	742	899	742	899	570	800
	875	908	911	878	911	878	810	800
	622	908	903	756	903	756	580	720
	622	681	699	766	699	766	720	740
	705	622	698	751	698	751	710	580

Anova: Two-Factor With Replication

SUM-MARY	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8	Total
<u>Green Thunder</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	9992	9236	10052	10727	10052	10727	6865	7535	75186
Average	999.2	923.6	1005.2	1072.7	1005.2	1072.7	686.5	753.5	939.825
Variance	18355.73	33149.16	3511.067	8995.344444	3511.067	8995.344	56378.06	6300.278	34406.96
<u>Green Forest</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	8972	7989	7574	7507	13774	7507	6540	7590	67453
Average	897.2	798.9	757.4	750.7	1377.4	750.7	654	759	843.1625
Variance	24971.07	27357.88	7490.044	5152.011111	3757250	5152.011	3515.556	2921.111	482030
<u>PIC Cos</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	8026	7478	7739	7747	7739	7747	6430	6990	59896
Average	802.6	747.8	773.9	774.7	773.9	774.7	643	699	748.7
Variance	16809.16	12324.18	8992.544	4226.233333	8992.544	4226.233	7734.444	5765.556	10295.5
<u>Total</u>									
Count	30	30	30	30	30	30	30	30	
Sum	26990	24703	25365	25981	31565	25981	19835	22115	
Average	899.6667	823.4333	845.5	866.033333	1052.167	866.0333	661.1667	737.1667	
Variance	25330.09	28242.67	19443.64	27893.41264	1233860	27893.41	21340.83	5409.799	

TABLE 4-continued

Source of Variation	ANOVA					
	SS	df	MS	F	P-value	F crit
Sample	1461215	2	730607.6	4.338013203	0.014219	3.03767
Columns	2780088	7	397155.5	2.358127411	0.024316	2.052154
Interaction	2453089	14	175220.6	1.040379891	0.414431	1.737799
Within	36378690	216	168419.9			
Total	43073082	239				

Table 5 below shows the leaf width of Green Thunder at harvest maturity as compared to Green Forest and PIC Cos. Leaf width is measured in centimeters. An analysis of variance was performed on the data and is shown below the data. As can be seen from the data in Table 5, Green Thunder is significantly wider in leaf width at harvest maturity than Green Forest but not significantly wider than PIC Cos.

TABLE 5

Location:	Leaf Width (cm) at Harvest Maturity							
	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
Green Thunder	20	21.5	21	18	21	18	22	20
	24	21	19	21	19	21	20	22
	23	21	21	18	18	18	20	20
	21	21	18	18	18	18	20	19
	19	16	21	20	21	20	22	19
	21.5	20	18	19	18	19	20	20
	16	19	18	18	18	18	22	22
	21	20	20	21	20	21	20	22
	23	20	18	19	17.5	19	22	22
	21	19	17	20	17	18	20	20
Green Forest	20.0	16.0	15.0	16.0	15.0	16.0	19.5	22.0
	20	20	14	17	14	17	19.5	19
	20	16	13	13	17	13	20	20
	18	19	17	16	17	16	20	21
	19	15	16	13	16	13	19	21
	18	21.5	13	14	13	14	19	19
	19	14	16	12	16	12	20	22
	20.5	16	12	14	12	14	19	22
	16	17	14	14	13.5	14	19	19
	21	18	13.5	15	14	15	19.5	20
PIC Cos	17	20	20	20	20	20	21	22
	19	19	20	16	19.5	16	20	22
	17	18	19	17	17	17	21	21
	15	17	17	19	17	19	22	22
	19	19	20	15	20	15	20	21
	17	16	16	17	16	17	22	19
	16	18	15	17	15	20	20	19
	14	21	17	20	17	20	20	19
	17	17	17	17	17	17	21	20
	17	15	17	17	17	17	21	21

Anova: Two-Factor With Replication

SUMMARY	Loc1	Loc2	Loc3	loc4	Loc5	Loc6	Loc7	Loc8	Total
<u>Green Thunder</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	209.5	198.5	191	192	187.5	190	208	206	1582.5
Average	20.95	19.85	19.1	19.2	18.75	19	20.8	20.6	19.78125
Variance	5.247222	2.558333	2.322222	1.511111111	2.069444	1.555556	1.066667	1.6	2.745847
<u>Green Forest</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	191.5	172.5	143.5	144	147.5	144	194.5	205	1342.5
Average	19.15	17.25	14.35	14.4	14.75	14.4	19.45	20.5	16.78125
Variance	2.225	5.513889	2.558333	2.488888889	2.958333	2.488889	0.191667	1.611111	8.378758
<u>PIC Cos</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	168	180	178	175	175.5	178	208	206	1468.5
Average	16.8	18	17.8	17.5	17.55	17.8	20.8	20.6	18.35625
Variance	2.4	3.333333	3.288889	2.722222222	2.913889	3.288889	0.622222	1.6	4.267049

TABLE 5-continued

Total								
Count	30	30	30	30	30	30	30	30
Sum	569	551	512.5	511	510.5	512	610.5	617
Average	18.96667	18.36667	17.08333	17.03333333	17.01667	17.06667	20.35	20.56667
Variance	6.050575	4.774713	6.691092	6.171264368	5.370402	6.202299	1.002586	1.495402

ANOVA							
Source of Variation	SS	df	MS	F	P-value	F crit	
Sample	360.3	2	180.15	74.37029958	2.67E-25	3.03767	
Columns	481.249	7	68.74985	28.38160993	1.59E-27	2.052154	
Interaction	211.4667	14	15.10476	6.235612923	2.1E-10	1.737799	
Within	523.225	216	2.422338				
Total	1576.241	239					

Table 6 below shows the leaf index of Green Thunder at harvest maturity as compared to Green Forest and PIC Cos. Leaf index is calculated by dividing the leaf length by the leaf width. An analysis of variance was performed on the data and is shown below the data. As can be seen in Table 6, Green Thunder has a significantly different leaf index than

Green Forest indicating that Green Thunder has a different leaf shape than Green Forest at harvest maturity. However, Green Thunder has a similar leaf index to PIC Cos indicating that Green Thunder has a similar leaf shape at harvest maturity to that of PIC Cos.

TABLE 6

Leaf Index at Harvest Maturity								
Location:	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
Green Thunder	1.35	1.33	1.33	1.39	1.33	1.4	1.32	1.5
	1.29	1.33	1.37	1.33	1.37	1.3	1.5	1.45
	1.3	1.33	1.33	1.39	1.39	1.4	1.45	1.55
	1.33	1.33	1.39	1.39	1.39	1.4	1.5	1.58
	1.37	1.44	1.33	1.35	1.33	1.4	1.36	1.58
	1.33	1.35	1.39	1.37	1.39	1.4	1.55	1.55
	1.44	1.37	1.39	1.39	1.39	1.4	1.36	1.36
	1.33	1.35	1.35	1.33	1.35	1.3	1.55	1.36
	1.3	1.35	1.39	1.37	1.43	1.4	1.45	1.41
	1.33	1.37	1.41	1.35	1.42	1.5	1.6	1.6
Green Forest	1.35	1.44	1.47	1.44	1.47	1.4	1.49	1.45
	1.35	1.35	1.5	1.41	1.5	1.4	1.54	1.58
	1.35	1.44	1.54	1.54	1.42	1.5	1.5	1.55
	1.39	1.37	1.53	1.44	1.41	1.4	1.5	1.43
	1.21	1.47	1.44	1.54	1.44	1.5	1.53	1.48
	1.39	1.33	1.54	1.5	1.54	1.5	1.58	1.58
	1.21	1.5	1.44	1.58	1.44	1.6	1.5	1.45
	1.34	1.44	1.58	1.5	1.58	1.5	1.58	1.36
	1.44	1.41	1.5	1.5	1.56	1.5	1.58	1.58
	1.33	1.39	1.56	1.47	1.5	1.5	1.59	1.5
PIC Cos	1.41	1.35	1.35	1.35	1.35	1.4	1.38	1.41
	1.37	1.37	1.35	1.44	1.36	1.4	1.4	1.36
	1.41	1.39	1.37	1.41	1.41	1.4	1.38	1.43
	1.53	1.41	1.41	1.37	1.41	1.4	1.36	1.45
	1.37	1.37	1.35	1.47	1.35	1.5	1.55	1.43
	1.41	1.44	1.44	1.41	1.44	1.4	1.36	1.58
	1.44	1.39	1.47	1.41	1.47	1.4	1.45	1.58
	1.5	1.33	1.41	1.35	1.41	1.4	1.5	1.58
	1.41	1.41	1.41	1.41	1.41	1.4	1.43	1.5
	1.41	1.47	1.41	1.41	1.41	1.4	1.48	1.48

Anova: Two-Factor With Replication

SUMMARY	Loc1	Loc2	Loc3	loc4	Loc5	Loc6	Loc7	Loc8	Total
<u>Green Thunder</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	13.37	13.55	13.68	13.66	13.79	13.9	14.64	14.94	111.53
Average	1.337	1.355	1.368	1.366	1.379	1.39	1.464	1.494	1.394125
Variance	0.00189	0.001139	0.000929	0.000604444	0.001166	0.003222	0.008738	0.008582	0.005698

TABLE 6-continued

Green Forest									
Count	10	10	10	10	10	10	10	10	80
Sum	13.36	14.14	15.1	14.92	14.86	14.8	15.39	14.96	117.53
Average	1.336	1.414	1.51	1.492	1.486	1.48	1.539	1.496	1.469125
Variance	0.005449	0.002916	0.002356	0.002795556	0.003582	0.004	0.001632	0.005716	0.006907
<u>PIC Cos</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	14.26	13.93	13.97	14.03	14.02	14.1	14.29	14.8	113.4
Average	1.426	1.393	1.397	1.403	1.402	1.41	1.429	1.48	1.4175
Variance	0.002671	0.001734	0.00169	0.001423333	0.001507	0.001	0.00421	0.006178	0.003039
<u>Total</u>									
Count	30	30	30	30	30	30	30	30	
Sum	40.99	41.62	42.75	42.61	42.67	42.8	44.32	44.7	
Average	1.366333	1.387333	1.425	1.420333333	1.422333	1.426667	1.477333	1.49	
Variance	0.004948	0.002413	0.005426	0.00438954	0.004129	0.004092	0.006703	0.006407	

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.235641	2	0.11782	37.63785199	9.47E-15	3.03767
Columns	0.354798	7	0.050685	16.19152694	4.53E-17	2.052154
Interaction	0.204919	14	0.014637	4.675831164	1.96E-07	1.737799
Within	0.67616	216	0.00313			
Total	1.471518	239				

Table 7 below shows the leaf area of Green Thunder at harvest maturity as compared to Green Forest and PIC Cos. Leaf area is calculated by multiplying the leaf length by the leaf width. An analysis of variance was performed on the data and is shown below the data. As can be seen in Table 7, Green Thunder has a significantly larger leaf area than both Green Forest and PIC Cos at harvest maturity.

TABLE 7

Leaf Area (cm2) at Harvest Maturity								
Location:	Loc1	Loc2	Loc3	loc4	Loc5	Loc6	Loc7	Loc8
Green Thunder	540	613	588	450	588	450	638	600
	744	588	484	588	494	588	600	704
	690	588	588	450	450	450	580	620
	588	588	450	450	450	450	600	570
	494	368	588	540	588	540	660	570
	613	540	450	494	450	494	620	620
	368	494	450	450	450	450	660	660
	588	540	540	588	540	588	620	660
	690	540	450	494	438	494	704	682
	588	494	408	540	408	486	640	640
Green Forest	540	368	330	368	330	368	566	704
	540	540	294	408	294	408	585	570
	540	368	260	260	408	260	600	620
	450	494	442	368	408	368	600	630
	437	330	368	260	368	260	551	651
	450	613	260	294	260	294	570	570
	437	294	368	228	368	228	600	704
	564	368	228	294	228	294	570	660
	368	408	294	294	284	294	570	570
	588	450	284	330	294	330	605	600
PIC Cos	408	540	540	540	540	540	609	682
	494	494	540	368	517	368	560	660
	408	450	494	408	408	408	609	630
	345	408	408	494	408	494	660	704
	494	494	540	330	330	330	620	630
	408	368	368	408	368	408	660	570
	368	450	330	408	330	540	580	570
	294	588	408	540	408	540	600	570
	408	408	108	108	108	108	630	600
	408	330	408	108	108	108	651	651

TABLE 7-continued

Anova: Two-Factor With Replication									
SUMMARY	Loc1	Loc2	Loc3	loc4	Loc5	Loc6	Loc7	Loc8	Total
<u>Green Thunder</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	5903	5353	4996	5044	4856	4990	6322	6326	43790
Average	590.3	535.3	499.6	504.4	485.6	499	632.2	632.6	547.375
Variance	11695.12	5092.9	4838.933	3174.044444	4150.933	3038.444	1321.289	2034.711	7410.668
<u>Green Forest</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	4914	4233	3128	3104	3242	3104	5817	6279	33821
Average	491.4	423.3	312.8	310.4	324.2	310.4	581.7	627.9	422.7625
Variance	5140.267	10016.46	4153.956	3246.933333	3859.067	3246.933	350.9	2672.1	18801.12
<u>PIC Cos</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	4035	4530	4144	3712	3525	3844	6179	6267	36236
Average	403.5	453	414.4	371.2	352.5	384.4	617.9	626.7	452.95
Variance	3683.833	6175.333	17438.04	24042.84444	21363.39	26864.71	1117.656	2345.789	22218.96
<u>Total</u>									
Count	30	30	30	30	30	30	30	30	
Sum	14852	14116	12268	11860	11623	11938	18318	18872	
Average	495.0667	470.5333	408.9333	395.3333333	387.4333	397.9333	610.6	629.0667	
Variance	12391.24	8927.361	14234.41	16244.50575	14238.46	16515.44	1333.076	2195.444	

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	676150.4	2	338075.2	47.43123725	8.39E-18	3.03767
Columns	2005861	7	286551.6	40.20258531	6.98E-36	2.052154
Interaction	280586.4	14	20041.89	2.811834592	0.000701	1.737799
Within	1539581	216	7127.691			
Total	4502179	239				

Table 8 below shows the core length of Green Thunder at harvest maturity as compared to Green Forest and PIC Cos. Core length is measured in centimeters. An analysis of variance was performed on the data and is shown below the

data. As can be seen in Table 8, Green Thunder has a significantly longer core than both Green Forest and PIC Cos at harvest maturity.

TABLE 8

Core Length (cm) at Harvest Maturity								
Location:	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
Green Thunder	7.7	9.2	5	6.5	5	6.5	5.5	7
	8.6	9	7	7	7	7	6	6.5
	8.4	10	7	6	7	6	6.5	6.5
	9	9.5	7	6	7	6	6.5	4
	8.2	5.5	6.5	7	6.5	7	7	4
	8	7.5	6	7	6	7	6	6.5
	7	7	6	5.5	6	5.5	5.8	6.8
	7.6	8	5.5	5	5.5	5	6.2	7
	9	6	6	6	6	6	7	7
	8	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Green Forest	7	5.5	5	5	5	5	6.5	6.2
	7	9	5	5	5	5	7	7
	8.2	7.8	5	5	5	5	6	6.5
	7.7	10	5	6	5	6	6.5	6.5
	9	6.5	5	5	5	5	6	6.2
	7	8	5	5	5	5	6.5	6
	9.5	6	5	5	5	5	6.5	6.5
	10	7	5	5	5	5	6.5	7
	5	6	5	5	5	5	7	7
	8.5	9	6	5	6	5	7	6.5

TABLE 8-continued

PIC Cos	7	6	5.5	6	5.5	6	5	6.5
	7.5	6	6	5	6	5	5	4.5
	6.5	6	5.5	5	5.5	5	4	4.2
	5	5	5	6	5	6	5	5.6
	7	6	6	5	6	5	4	5
	6	7	5	6	5	6	4	5
	6	7	5	5	5	5	5	5
	4	8	4	6	4	5	5	4
	5.5	5	5	5	5	5	4	4
	5.5	5	5	5	4	4	5	4

Anova: Two-Factor With Replication

SUMMARY	Loc1	Loc2	Loc3	loc4	Loc5	Loc6	Loc7	Loc8	Total
<u>Green Thunder</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	81.5	78.2	62.5	62.5	62.5	62.5	63	61.8	534.5
Average	8.15	7.82	6.25	6.25	6.25	6.25	6.3	6.18	6.68125
Variance	0.398333	2.457333	0.458333	0.458333333	0.458333	0.458333	0.242222	1.368444	1.299264
<u>Green Forest</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	78.9	74.8	51	51	51	51	65.5	65.4	488.6
Average	7.89	7.48	5.1	5.1	5.1	5.1	6.55	6.54	6.1075
Variance	2.167667	2.315111	0.1	0.1	0.1	0.1	0.136111	0.129333	1.789563
<u>PIC Cos</u>									
Count	10	10	10	10	10	10	10	10	80
Sum	60	61	52	54	51	52	46	47.8	423.8
Average	6	6.1	5.2	5.4	5.1	5.2	4.6	4.78	5.2975
Variance	1.111111	0.988889	0.344444	0.266666667	0.488889	0.4	0.266667	0.668444	0.764804
<u>Total</u>									
Count	30	30	30	30	30	30	30	30	
Sum	220.4	214	165.5	167.5	164.5	165.5	174.5	175	
Average	7.346667	7.133333	5.516667	5.583333333	5.483333	5.516667	5.816667	5.833333	
Variance	2.090851	2.36023	0.560057	0.501436782	0.629023	0.577299	0.976609	1.2685057	

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	77.33475	2	38.66737	58.06275418	6.61E-21	3.03767
Columns	121.8153	7	17.40218	26.13104102	9.34E-26	2.052154
Interaction	38.77458	14	2.769613	4.158838409	1.94E-06	1.737799
Within	143.847	216	0.665958			
Total	381.7716	239				

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Table 9 below shows the length of the fourth true leaf of Green Thunder as compared to Green Forest and PIC Cos. Leaf length is measured in centimeters of a 20-day old seedling. An analysis of variance was performed on the data

and is shown below the data. As can be seen in Table 9, Green Thunder has a significantly shorter fourth leaf length than Green Forest but about the same fourth leaf length as PIC Cos.

TABLE 9

4th Leaf Length (cm)		
Green Thunder	Green Forest	PIC Cos
3.8	5.6	3.8
4.3	5.5	3.9
3.9	4.8	3.6
4.1	5.8	4.2
3.9	4	3.7
4.4	4.8	3.8
4.6	5.5	3.9
3.5	5.5	4
4.1	5.6	4.1
4.3	5.8	4

TABLE 9-continued

Groups	Count	Sum	Average	Variance
Green Thunder	15	61	4.066666667	0.075238
Green Forest	15	78.8	5.253333333	0.321238
PIC Cos	15	59.4	3.96	0.046857

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	15.46133333	2	7.730666667	52.31278	3.96E-12	3.219938
Within Groups	6.206666667	42	0.147777778			
Total	21.668	44				

Table 10 below shows the width of the fourth true leaf of Green Thunder as compared to Green Forest and PIC Cos. Leaf width is measured in centimeters of a 20-day old seedling. An analysis of variance was performed on the data and is shown below the data. As can be seen in Table 10, Green Thunder has a significantly narrower fourth leaf width than Green Forest but about the same fourth leaf width as PIC Cos.

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Table 11 below shows the leaf index of the fourth true leaf of Green Thunder as compared to Green Forest and PIC Cos. Leaf index is calculated by dividing the leaf length by the leaf width of the fourth true leaf of a 20-day old seedling. An analysis of variance was performed on the data and is shown below the data. As can be seen in Table 11, Green Thunder has a significantly different leaf index than both Green Forest and PIC Cos indicating that Green Thunder has a

TABLE 10

Green Thunder	Green Forest	PIC Cos
2.6	2.8	2.3
2.8	3.2	2.5
2.5	2.7	2.2
2.6	3	2.5
2.4	2.1	2.1
2.5	2.6	2.4
2.6	2.8	2.3
2.2	2.9	2.5
2.6	2.9	2.5
2.8	3	2.4
2.6	3	2.4
2.5	2.4	2.2
2.5	2.5	2.4
2.7	3	2.5
2.8	3	2.6

Groups	Count	Sum	Average	Variance
Green Thunder	15	38.7	2.58	0.026
Green Forest	15	41.9	2.793333333	0.083524
PIC Cos	15	35.8	2.386666667	0.01981

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.241333333	2	0.620666667	14.39691	1.73E-05	3.219938
Within Groups	1.810666667	42	0.043111111			
Total	3.052	44				

different fourth true leaf shape than either Green Forest or PIC Cos.

TABLE 11

4th Leaf Index		
Green Thunder	Green Forest	PIC Cos
1.46	2.07	1.65
1.54	1.72	1.56
1.56	1.78	1.64
1.58	1.93	1.68
1.63	1.9	1.76
1.76	1.85	1.58
1.77	1.96	1.7
1.59	1.9	1.6
1.58	1.93	1.64
1.54	1.93	1.67
1.46	1.97	1.63
1.6	1.88	1.73
1.71	1.84	1.67
1.56	1.8	1.64
1.43	1.83	1.73

Anova: Single Factor
SUMMARY

Groups	Count	Sum	Average	Variance
Green Thunder	15	23.77	1.584666667	0.010241
Green Forest	15	28.29	1.886	0.007526
PIC Cos	15	24.88	1.658666667	0.003155

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.739791111	2	0.369895556	53.03947	3.22E-12	3.219938
Within Groups	0.292906667	42	0.006973968			
Total	1.032697778	44				

Table 12 below shows the cotyledon length of Green Thunder as compared to Green Forest and PIC Cos. Cotyledon length was measured in millimeters. An analysis of variance was performed on the data and is shown below the

data. As can be seen in Table 12, Green Thunder has a significantly shorter cotyledon length than Green Forest but similar cotyledon length to PIC Cos.

TABLE 12

Cotyledon length (mm)		
Green Thunder	Green Forest	PIC Cos
15	19	14
15	20	14
15	19	14
16	19	12
16	20	14
15	18	14
13	18	14
13	19	16
15	18	16
15	19	16
15	19	14
15	19	14

Anova: Single Factor
SUMMARY

Groups	Count	Sum	Average	Variance
Green Thunder	12	178	14.83333	0.878788
Green Forest	12	227	18.91667	0.44697
PIC Cos	12	172	14.33333	1.333333

TABLE 12-continued

Source of Variation	ANOVA					
	SS	df	MS	F	P-value	F crit
Between Groups	151.7222222	2	75.86111	85.58689	8.72E-14	3.284924
Within Groups	29.25	33	0.886364			
Total	180.9722222	35				

Table 13 below shows the cotyledon width of Green Thunder as compared to Green Forest and PIC Cos. Cotyledon width was measured in millimeters. An analysis of variance was performed on the data and is shown below the data. As can be seen in Table 13, Green Thunder has a significantly wider cotyledon width than both Green Forest and PIC Cos.

TABLE 13

Cotyledon Width (mm)		
Green Thunder	Green Forest	PIC Cos
10	9	9
10	9	8
10	9	9
10	9	8
10	9	8
11	9	8
9	9	8
9	9	8
10	9	9
10	9	9
10	9	8
10	9	8

Anova: Single Factor
SUMMARY

Groups	Count	Sum	Average	Variance
Green Thunder	12	119	9.916667	0.265152
Green Forest	12	108	9	0
PIC Cos	12	100	8.333333	0.242424

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	15.16666667	2	7.583333	44.8209	3.92E-10	3.284924
Within Groups	5.583333333	33	0.169192			
Total	20.75	35				

Table 14 below shows the cotyledon index of Green Thunder as compared to Green Forest and PIC Cos. Cotyledon index is calculated by dividing the cotyledon leaf length by the cotyledon leaf width. An analysis of variance was performed on the data and is shown below the data. As

can be seen in Table 14, Green Thunder has a significantly different cotyledon index than both Green Forest and PIC Cos indicating that Green Thunder has a different cotyledon leaf shape than both Green Forest and PIC Cos.

TABLE 14

Cotyledon Width (mm)		
Green Thunder	Green Forest	PIC Cos
1.5	2.11	1.56
1.5	2.22	1.75
1.5	2.11	1.56
1.6	2.11	1.5

TABLE 14-continued

1.6	2.22	1.75
1.36	2	1.75
1.44	2	1.75
1.44	2.11	1.75
1.5	2	1.78
1.5	2.11	1.78
1.5	2.11	1.75
1.5	2.11	1.75

Anova: Single Factor				
SUMMARY				
Groups	Count	Sum	Average	Variance
Green Thunder	12	17.94	1.495	0.004227
Green Forest	12	25.21	2.100833	0.005408
PIC Cos	12	20.43	1.7025	0.009948

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.275038889	2	1.137519	174.2583	2.89E-18	3.284924
Within Groups	0.215416667	33	0.006528			
Total	2.490455556	35				

DEPOSIT INFORMATION

A deposit of the Synergene Seed & Technology, Inc. Proprietary lettuce cultivar designated Green Thunder disclosed above and recited in the appended claims has been made with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110. The date of deposit was Aug. 14, 2007. The deposit of 2,500 seeds was taken from the same deposit maintained by Synergene Seed & Technology, Inc. since prior to the filing date of this application. All restrictions upon the deposit have been removed, and the deposit is intended to meet all of the requirements of 37 C.F.R. 1.801-1.809. The ATCC accession number is PTA-8605. The deposit will be maintained in the depository for a period of 30 years, or 5 years after the last request, or for the effective life of the patent, whichever is longer, and will be replaced as necessary during that period.

While a number of exemplary aspects and embodiments have been discussed above, those of skill in the art will recognize certain modifications, permutations, additions and sub-combinations thereof. It is therefore intended that the following appended claims and claims hereafter introduced are interpreted to include all such modifications, permutations, additions and sub-combinations as are within their true spirit and scope.

What is claimed is:

1. A seed of lettuce cultivar Green Thunder, wherein a representative sample of seed of said cultivar was deposited under ATCC Accession No. PTA-8605.

2. A lettuce plant, or a part thereof, produced by growing the seed of claim 1.

3. A tissue culture of cells produced from the plant of claim 2, wherein said cells of the tissue culture are produced from a plant part selected from the group consisting of leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, anther, pistil, flower, and stem.

4. A protoplast produced from the plant of claim 2.

5. A protoplast produced from the tissue culture of claim 3.

6. A lettuce plant regenerated from the tissue culture of claim 3, wherein the plant has all the morphological and physiological characteristics of cultivar Green Thunder.

7. A method for producing an F₁ hybrid lettuce seed, wherein the method comprises crossing the plant of claim 2 with a different lettuce plant and harvesting the resultant F₁ hybrid lettuce seed.

8. A hybrid lettuce seed produced by the method of claim 7.

9. A hybrid lettuce plant, or a part thereof, produced by growing said hybrid seed of claim 8.

10. A method for producing a male sterile lettuce plant wherein the method comprises transforming the lettuce plant of claim 2 with a nucleic acid molecule.

11. A male sterile lettuce plant produced by the method of claim 10.

12. A method for producing an herbicide resistant lettuce plant wherein the method comprises transforming the lettuce plant of claim 2 with a transgene wherein the transgene confers resistance to an herbicide selected from the group consisting of imidazolinone, sulfonylurea, glyphosate, glufosinate, L-phosphinothricin, triazine and benzonitrile.

13. An herbicide resistant lettuce plant produced by the method of claim 12.

14. A method of producing an insect resistant lettuce plant wherein the method comprises transforming the lettuce plant of claim 2 with a transgene that confers insect resistance.

15. An insect resistance lettuce plant produced by the method of claim 14.

16. The lettuce plant of claim 15 wherein the transgene encodes a *Bacillus thuringiensis* endotoxin.

17. A method of producing a disease resistant lettuce plant wherein the method comprises transforming the lettuce plant of claim 2 with a transgene that confers disease resistance.

18. A disease resistant lettuce plant produced by the method of claim 17.

19. A method of producing a lettuce plant with a value-added trait, wherein the method comprises transforming the lettuce plant of claim 2 with a transgene encoding a protein selected from the group consisting of a ferritin, a nitrate reductase and a monellin.

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20. A lettuce plant with a value-added trait produced by the method of claim 19.

21. A lettuce plant, or a part thereof, having all the physiological and morphological characteristics of the cultivar Green Thunder, wherein a representative sample of seed of said cultivar was deposited under ATCC Accession No. PTA-8605.

22. A method of introducing a desired trait into lettuce cultivar Green Thunder wherein the method comprises:

- a. crossing a Green Thunder plant, wherein a representative sample of seed was deposited under ATCC Accession No. PTA-8605, with a plant of another lettuce cultivar that comprises a desired trait to produce progeny plants, wherein the desired trait is selected from the group consisting of male sterility, herbicide resistance, insect resistance, and resistance to bacterial disease, fungal disease, or viral disease;
- b. selecting progeny plants that have the desired trait to produce selected progeny plants;
- c. crossing the selected progeny plants with the Green Thunder plants to produce backcross progeny plants;
- d. selecting for backcross progeny plants that have the desired trait and physiological and morphological characteristics of lettuce cultivar Green Thunder listed in Table 1; and

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e. repeating steps (c) and (d) three or more times in succession to produce selected fourth or higher backcross progeny plants that comprise the desired trait and all of the physiological and morphological characteristics of lettuce cultivar Green Thunder listed in Table 1.

23. A plant produced by the method of claim 22, wherein the plant has the desired trait and all of the physiological and morphological characteristics of lettuce cultivar Green Thunder listed in Table 1.

24. The plant of claim 23, wherein the desired trait is herbicide resistance and the resistance is conferred to an herbicide selected from the group consisting of imidazolinone, sulfonylurea, glyphosate, glufosinate, L-phosphinothricin, triazine and benzonitrile.

25. The plant of claim 23, wherein the desired trait is insect resistance and the insect resistance is conferred by a transgene encoding a *Bacillus thuringiensis* endotoxin.

26. The plant of claim 23, wherein the desired trait is male sterility and the trait is conferred by a nucleic acid molecule.

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